DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Industrial Application]This invention relates to prevention and the treating agent of osteoclasis nature bone diseases, such as malignant hypercalcemia, a Paget's disease of bone, or osteoporosis.

[0002]

[Description of the Prior Art]In recent years, what is called geriatric diseases are increasing with the rapid increase in an elderly population. Especially, bone diseases including osteoporosis occur fracture frequently, and development of the prevention and cure is desired as an illness which leads to a bedridden elderly.

[0003]Once a bone is formed, after not the structure that does not change at all but osteogenesis, and osteoclasis balance, the structure and quantity are maintained. Therefore, if the balance collapses by the cause of aging or others, the symptoms of various bone diseases will be shown.

[0004]Although the malignant hypercalcemia to which myeloma, a lymphoma, etc. happen owing to, the Paget's disease of bone brought about by locality osteoclasis, and the cause are unknown as a disease which occurs by the gastric upset of osteoclasis, the osteoporosis etc. in which bone quantity decreases by aging are mentioned.

[0005]A bone consists of calcium salt which is the collagen fiber and minerals which are mainly quality of organicity, these both are connected, and the bone which is a firm structure strong against tension and a pressure is formed. Although calcium salt occupies 70% of total bone weight, in the bone disease of osteoclasis nature like osteoporosis, with the advance, calcium salt is eluted in blood from a bone, with a hypercalcemia, calcium salt is lost gradually and it especially goes from a bone.

[0006]The method with which the method of maintaining calcium compensates calcium again at a therapy was adopted as prevention of such a disease, and active-vitamin-D_3 pharmaceutical preparation, a calcium preparation, etc. have so far been used. The estrogen preparation and a hormone drug like calcitonin preparation have been used in order to control deliming from a bone.

[0007]

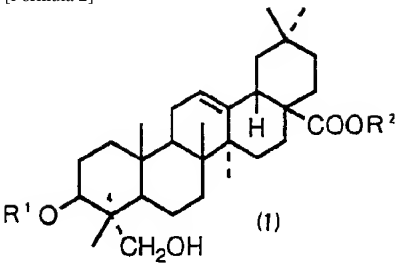
[Problem(s) to be Solved by the Invention]however, although a certain amount of [the effect of these drugs] effect to mitigation of a pain and prevention of progression of condition of disease is accepted, the effect is not satisfactory and the medicinal properties which show a more positive effect are called for. When taking in the ingredient concerned daily for the purpose of prevention of an absorptivity bone disease, an ingestion is desirable and it is desired for the safety which can especially be taken in with ingesta as a combination ingredient of ingesta to be high.

[0008]

[Means for Solving the Problem]This invention persons got to know that calcium from a culture new-born-mouse calvarium and inorganic-phosphoric-acid isolation which PTHrp (parathyroid hormone associated protein) or its active fragment induces had a mechanism of an absorptivity bone disease like osteoporosis, and high correlation previously. And when it was searching for prevention and a curative effect over an absorptivity bone disease about various compounds using this technique, by this evaluation system, a hederagenin compound found out that isolation depressant action of calcium and inorganic phosphoric acid was shown, and completed this invention.

[0009]namely, this invention -- formula (1)

[0010]
[Formula 2]



[0011](R^1 and R^2 show a hydrogen atom or a glycosyl group independently among a formula.) -- prevention and the treating agent of the bone disease which makes an active principle the hederagenin compound expressed are provided.

[0012]The hederagenin compound used for this invention is hederagenin. [The compound both R^1 and whose R^2 are hydrogen atoms in a formula (1)] And the hederagenin glycoside which the hydrogen atom of R^1 and/or R^2 replaced by various glycosyl groups in the formula (1) is meant, For example, alpha-hederin which is a saponin component of Araliaceae KIZUTA (Hedera tobleri), Hederagenin glycosides, such as the KAROPANAKKUSU saponin A and B (No. Chem.Pharm.Bull. 37-volume 2 311page 1989) obtained from Araliaceae Kalopanax (Kalopanax pictus), can be illustrated.

[0013]As shown in the after-mentioned example, in order for the hederagenin itself which is aglycon to show osteoclasis depressant action, it is hederagenin 3-arabino pyranoside, for example. [In a formula (1), R^1 is alpha-arabinopyranosyl group and R^2 abbreviates to a compound and following Hed-3-ara which is a hydrogen atom. A partial hydrolysate of a natural product like], various hederagenin glycosides which introduced various glycosyl groups into R^1 and/or R^2 by various glycosyltransferase reactions etc., etc. can be used effective in this invention.

[0014]In order for these compounds to show the activity of osteoclasis control, It is indispensable to the 4th place to have a hydroxymethyl group at a configuration of R, for example, as shown in the after-mentioned example, although oleanolic acid which is 4-dimethyl object has the completely same structure as hederagenin, it does not show osteoclasis depressant action other than a substituent of the 4th place.

[0015]A hederagenin compound used for this invention, for example Araliaceae KIZUTA (Hedera tobleri), Araliaceae Kalopanax (Kalopanax pictus), the Lardizabalaceae akebi (Akebia quinata), Compositae Sion (Aster tataricus), Ranunculaceae top Clematis maximowicziana (Clematis mandshurica), ICHIRINZAKISENNINSOU (Clematis brachyura), They may be the synthetic compounds compounded by publicly known method also with a natural product refined from plant bodies, such as Pittosporaceae TOBERA (Pittosporum tobira) and the Sapindaceae Sapindus mukurossi (Sapindusmukurossi). When using a natural product, it is not necessary to necessarily refine even in a pure article, and it is a range which does not spoil an effect of this invention, and a mixture of an extract,

partially purified substance, and various hederagenin compounds, etc. can be used.
[0016]There is no knowledge that these compounds show osteoclasis depressant action and are effective in absorptivity bone diseases, such as osteoporosis, although a hederagenin compound used for this invention is a known compound obtained as a plant component.
[0017]A hederagenin compound used for this invention can also be used combining various hederagenin compounds as an active principle, although it may use independently.
[0018]What is necessary is to make the above-mentioned hederagenin compound into an active principle, and just to pharmaceutical-preparation-ize combining a publicly known carrier for drugs in accordance with a conventional method, in order to manufacture prevention and a treating agent of an absorptivity bone disease of this invention.
[0019]In accordance with a conventional method, administration by various pharmaceutical forms is possible for an active principle of this invention. For example, as an orally administered drug, a capsule, a tablet, a granule, subtle granules, syrups, dry-syrups, etc. can be illustrated, and percutaneous absorption agents, such as nasally administered drugs, such as suppositories, such as a suppository, vagina suppository, etc. besides injections, and a spray, ointment, and a percutaneous absorption tape, can be illustrated as a parenteral administration agent.
[0020]It is also possible to add in the usual ingesta and to take in an active principle of this invention daily. Although there is no limitation in particular in a kind of foodstuffs which can be added, since there is characteristic flavor in an active principle of this invention, it is preferred to add about 0.01 to 1 % of the weight which is a range which impairs an original taste of foodstuffs, for example for neither drinks, such as drinkable preparations, nor strong foodstuffs of sweet taste, such as a candy.
[0021]What is necessary is just to usually prescribe 3 - 30 mg/kg for the patient in 1 to 3 steps 1-100mg/kg adult 1 sunny as the sum total of an active principle preferably, in administering an active principle of this invention orally. Intake in a case of adding to ingesta etc. and taking in an active principle of this invention daily also applies to a dose in internal use. These can be suitably fluctuated according to age, condition, etc.
[0022]In prescribing an active principle of this invention for the patient parenterally, What is necessary is for what is necessary to be to take into consideration blood drug concentration of an active principle, and just to consider it as about about 1/10 dose in internal use, and just to usually prescribe 0.3 - 3.0 mg/kg for the patient in 1 to 3 steps 0.1-10mg/kg adult 1 sunny as the sum total of an active principle preferably. These doses can be suitably fluctuated according to age, condition, etc.
[0023]An active principle of this invention is an ingredient of a medicinal herb used from ancient times more widely, and a problem is not observed in toxicity at all in a regular amount of internal use. Although hemolysis is reported about alpha-hederin which is a kind of an active principle of this invention (nature of bioactive natural product 419 page Ishiyaku Publishers 1978), since it is known that this hemolysis will disappear by removal of a glycosyl group, It is desirable to use hederagenin which is the aglycon in menstrual blood pipe administration.
[0024]Next, a valuation method of isolation depressant action of the calcium and inorganic phosphoric acid is explained about a hederagenin compound which is an active principle of this invention.
[0025]PTHrp (1-34) which PTHrp is the protein identified as a human hypercalcemia inducement factor, and is the fragmentation which consists of these one to 34th amino acid residue is an active type. And this PTHrp (1-34), Since an osteoclasis promotion operation is shown by in vitro one (a journal OBU clinical investigation, 81 volumes, No. 2, 596 - 600 pages, 1988; endocrinology, 123 volumes, 2841 -

2848 pages, 1988), It is possible to use it as an evaluation system of osteoporosis.
[0026]Then, evaluation of in vitro osteoclasis depressant action of this invention active principle was performed using a culture new-born-mouse calvarium and PTHrp (1-34). This evaluation system is what added improvement to a method (a journal OBU clinical investigation, 44 volumes, No. 1, 103 - 116 pages, 1965) of Lois who uses an antebrachial bone and ^{45}Ca of an embryo rat, It has an advantage which can evaluate many samples safely, without using radioisotope.
[0027]This improving method, To a bone disease treating agent, already as a screening system. Although it is the method (the Japanese Society for Bone and Mineral Metabolism magazine, eight volumes, No. 3, 221 pages, 1990; said 9 volume, No. 3, 239 pages, 1991; Japanese journal OBU pharmacology, 55 volumes and the supplement No. 1, 120 pages, 1991) used widely, It will be as follows if the outline is explained.
[0028]Namely, after extracting and carrying out preculture of the calvarium of a 4 - 6 age-in-day ICR system mouse for one day, It is the method of exchanging for a culture medium which added PTHrp (1-34) of a sample which should be authorized, and $10^{-8}M$, cultivating for two more days, measuring calcium and inorganic phosphorus concentration after an end of culture, and in a culture supernatant, and comparing with an addition group of only PTHrp (1-34).
[0029]When this method estimated osteoclasis depressant action, as shown in the after-mentioned example, as for a group which added a compound of this invention, it became clear that calcium and inorganic phosphorus concentration in a culture supernatant were falling compared with a processing group of only PTHrp (1-34).
[0030]
[Function]The hederagenin compound which is an active principle of this invention has the operation which controls calcium from a culture new-born-mouse calvarium and inorganic-phosphoric-acid isolation which PTHrp (1-34) which is parathyroid hormone related peptide causes, as shown in the after-mentioned example.
[0031]Thus, since the hederagenin compound used as an active principle by this invention has the operation which controls calcium and inorganic-phosphoric-acid isolation, it is conjectured to prevent and treat effectively absorptivity bone diseases, such as malignant hypercalcemia, a Paget's disease of bone, and osteoporosis.
[0032]
[Example]Subsequently, although this invention is explained still in detail based on an example, this invention is not limited to these examples.
[0033]
10 g of roots (it receives from a dry article and a Chinese medicine second-class drug seller) of refining Kalopanax of the hederagenin compound from reference example 1. Kalopanax were judged finely, and it filtered after immersion for two days to 200 ml of methanol 50% at the room temperature. 200 ml of methanol was again added to residue 50%, and also it filtered after immersion for two days at the room temperature. 200 ml of methanol was again added to residue 50%, and it filtered after immersion similarly. The filtrate obtained by three extraction was collected, vacuum concentration back-freeze-dried, and 170 mg of extracts were obtained.
[0034]This extract was made to stick to ethyl acetate after **** at a SEPPAK C_{18} cartridge (made by Millipore RIMITTEDO), and it was eluted with 10 ml of methanol 80% after 10 ml of methanol 60%.
[0035]Give the obtained 80% methanol elution classification to the high performance chromatography

using a Delvelosil ODS column (30 cm and the 2-cm x Nomura chemicals company make), and it is eluted to isocratic one with 60% acetonitrile / water, 24 mg of alpha-hederin and 6 mg of Hed-3-ara were obtained. Hederagenin was obtained by hydrolyzing obtained alpha-hederin.

[0036]Among the purification process, the structural analysis of the compound was conducted by comparing spectrum data with a literature value, and used the refined material produced by making it the same for future bioassays and pharmaceutical preparation. The oleanolic acid compared by the bioassay used the commercial (made by a sigma company) thing.

[0037]After cutting down the calvarium of the measurement ICR system mouse (four to 6 age in day) of depressor effect to calcium from a culture new-born-mouse calvarium and inorganic phosphorus isolation which were caused by example 1.PTHrp (1-34) and removing tissue, punch-out is carried out to 4 mm in diameter. About this, it is a 5%FBS-BGJb culture medium. It puts one piece at a time into 48 hole plate containing [the BGJb culture medium (made by Fitton-Jackson modification/Sigma) which contains fetal calf serum 5%], and preculture is carried out under the condition of 37 **, and 5% carbon dioxide / air for 24 hours. It exchanges for the 5%FBS-BGJb culture medium containing the sample of the concentration shown in PTHrp (1-34) and the table of 10^{-8}M after the end of preculture, and cultivates for further 48 hours.

[0038]After the end of culture, the calcium concentration of the culture supernatant was measured with the OCPC method, inorganic phosphorus concentration was measured by molybdic acid direct method using VX1000 type biochemical automatic analyzer by JEOL Co., Ltd., and only PTHrp (1-34) considered the osteoclasis operation as compared with the value of the control group of addition. This result is shown in Table 1.

[0039]

[Table 1]

ヘデラゲニン化合物の新生仔マウス頭蓋冠における骨吸収抑制作用

化合物	添加濃度（μg/ml）	PTHrp処理	Ca濃度(mg/dl) 平均値	(%)	無機P濃度(mg/dl) 平均値	(%)
コントロール	−	あり	13.12	(100)	4.26	(100)
	−	なし	10.83	(83)	3.40	(80)
α−ヘデリン	0.3	あり	11.90	(91)	3.97	(93)
	1	あり	10.08	(77)	3.12	(73)
	3	あり	9.55	(73)	2.85	(70)
	10	あり	9.01	(69)	2.85	(70)
Hed-3-ara	0.3	あり	11.99	(91)	3.93	(92)
	1	あり	11.83	(90)	3.73	(88)
	3	あり	11.26	(86)	3.68	(86)
	10	あり	10.62	(81)	3.39	(80)
ヘデラゲニン	0.3	あり	11.01	(84)	3.47	(81)
	1	あり	12.01	(92)	3.92	(92)
	3	あり	11.51	(88)	3.64	(85)
	10	あり	10.05	(77)	3.14	(74)
オレアノール酸	0.3	あり	12.15	(93)	3.97	(93)
	1	あり	12.64	(96)	4.19	(98)
	3	あり	12.58	(96)	4.00	(94)
	10	あり	12.35	(94)	3.97	(93)

N＝2の平均値

[0040]Each hederagenin compound of this invention controlled isolation of calcium and inorganic phosphoric acid so that clearly from the result of Table 1. On the other hand, other than the substituent of the 4th place, although the oleanolic acid which is 4-dimethyl object had the completely same structure as hederagenin, it did not show such an operation.

[0041]That is, from the result of Table 1, in order for these hederagenin compounds to show the activity of osteoclasis control, it was judged to be indispensable to have a hydroxymethyl group by the configuration of R in the 4th place.

[0042]It was suggested strongly that it is that in which the hederagenin compound of this invention can prevent or control malignant hypercalcemia, and it became clear from the above-mentioned result that the compound of this invention is useful as prevention and a treating agent of a bone disease.

[0043]Manufacture of an example 2. capsule [Formula]

alpha-hederin 100 copies (weight section)

Potato starch 148 copy ** magnesium stearate Two copy ** [0044][Process] After mixing the above-mentioned ingredient well with a grinding machine according to a formula, it filled up the No. 1 hard gelatine capsule with 250 mg at a time, and the capsule which contains 100 mg of alpha-hederin among

1 capsule was obtained.
[0045]the manufacture witepsol H-15 of an example 3. rectal suppository -- warming -- it dissolved, it added so that it might become the concentration of 12.5mg/ml about hederagenin at this, and it mixed uniformly, and the rectal suppository which pours this in 2 ml at a time subsequently to a rectal suppository mold, cools, and contains 25 mg of hederagenin among 1 agent was manufactured.
[0046]Manufacture of example 4. drinkable preparations [Formula]
alpha-hederin 2gDL-sodium tartrate 10-mg succinic acid 1-mg liquid sugar 80g citrate 1.2g vitamin C 1g perfume 1-ml potassium chloride 0.1g magnesium sulfate 50 mg [0047][Process] After having dissolved the above-mentioned ingredient in 800 ml of distilled water according to the formula, adding distilled water and considering it as 1000 ml of whole quantity, it sterilized by a 0.22-micrometer sterilization filter, aseptic [of every 100 ml] was carried out to the amber bottle, and the drinkable preparations containing alpha-hederin of 200 mg of 1 agent hits were obtained.
[0048]Manufacture of an example 5. candy [Formula]
alpha-hederin 1g purified water 1g granulated sugar 49g starch syrup 48g citrate 0.5g lemon perfume 0.5g [0049][Process] In accordance with the conventional method, heating fusion of granulated sugar and the starch syrup was carried out, after suspending and adding other ingredients to purified water, it mixed uniformly and one-grain a 2-g candy was manufactured. 20 mg of alpha-hederin is contained in one grain.
[0050]
[Effect of the Invention]Since the hederagenin compound which is an active principle of this invention controlled calcium from a culture new-born-mouse calvarium and inorganic phosphorus isolation which were caused by PTHrp (1-34), inhibiting an osteoclasis operation was checked. And these active principles are ingredients of the medicinal herb used from ancient times more widely.
In safety, there is no point in particular that becomes a problem.

[0051]Therefore, prevention and the treating agent of the absorptivity bone disease of this invention are useful for prevention or the therapies of an absorptivity bone disease, such as malignant hypercalcemia, a Paget's disease of bone, and osteoporosis. It is useful also as foodstuffs for health for prevention of an absorptivity bone disease, etc. by adding to ingesta and taking in the active principle of this invention daily.